

PATENT

ATTORNEY DOCKET NO. CGNE.023.08US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Goodman, *et al.*

) Examiner: Not Yet Assigned

Serial No.: Not Yet Assigned

)
) Art Unit: Not Yet Assigned

Filed: November 23, 1999

)
) TRANSMITTAL OF
) CONTINUATION UNDER 37 C.F.R.
) § 1.53(b)

For: MOLECULAR FARMING

BOX PATENT APPLICATION

Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

This is a request for filing a patent application under 37 C.F.R. § 1.53(b) in the name of
inventor: **GOODMAN, *et al.***

For: MOLECULAR FARMING

This application is a ☒ Continuation [] Divisional [] Continuation-in-part of prior
Application No.: 08/855,260 filed May 13, 1997 from which priority under 35 U.S.C. § 120 is
claimed.

Application Elements:

22 Pages of Specification, Claims and Abstract

0 Sheets of formal Drawings

CERTIFICATE OF EXPRESS MAILING

"Express Mail" Label No.: EL398977989US

Date of Deposit: 11/23/99

I hereby certify under 37 C.F.R. 1.10 that this correspondence is
being deposited with the United States Postal Service as "Express
Mail Post Office to Addressee" with sufficient postage on the date
indicated above and is addressed to the Commissioner of Patents
and Trademarks, Washington, D.C., 20231.

Jacob Zweig
(Signature)

JACOB ZWEIF
(Printed Name)

- ☒ Declaration
[] Unexecuted Combined Inventor Declaration and Power of Attorney
☒ Copy from prior application (37 CFR 1.63(d) for a continuation or divisional).

The entire disclosure of the prior application from which a copy of the declaration is herein supplied is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

[] Deletion of inventors Signed statement attached deleting inventor(s) named in the prior application, *see* CFR 1.63(d)(2) and 1.33(b).

Accompanying Application Parts:

- [] Assignment and Assignment Recordation Cover Sheet (recording fee of \$40.00 enclosed)
☒ Power of Attorney
[] 37 CFR 3.73(b) Statement by Assignee
☒ Information Disclosure Statement with Form 1449
[] Copies of IDS Citations
☒ Preliminary Amendment
☒ Return Receipt Postcard
[] Small Entity Statement(s) [] Statement filed in prior application. Status still proper and desired.
[] Other:
[] A sequence listing.
[] paper copy.
[] computer readable copy.
[] Statement in Compliance with Requirements for Patent Applications Containing Nucleotide and/or Amino Acid Sequence.

Claim For Foreign Priority

- [] Priority of _____ Application No. _____ filed on _____ is claimed under 35 U.S.C. § 119
[] The certified copy has been filed in prior application U.S. Application No. _____
[] the certified copy will follow.

Extension of Time for Prior Pending Application

- [] A Petition for Extension of Time is being concurrently filed in the prior pending application. A copy of the Petition for Extension of Time is attached.

Amendments

- ☒ Amend the specification by inserting before the first line the sentence: "This is a

☒ Continuation ☐ Continuation-in-part ☐ Divisional application of copending application of 08/855,260 filed May 13, 1997, which is a continuation of 08/463,182 filed June 5, 1995, now USPN 5,629,175, which is a continuation of 08/164,346 filed December 8, 1993, now USPN 5,550,038, which is a continuation of 07/507,380 filed April 9, 1990, abandoned, which is a continuation 06/760,236 filed July 29, 1985, now USPN 4,956,282, which disclosures are incorporated herein by reference.

- ☐ Application No. _____ filed on _____.
- ☐ International Application _____ filed on _____, which designated the United States, disclosure of which is incorporated herein by reference."
- ☒ Cancel in this application original claims 2-13 of the prior application before calculating the filing fee

Fee Calculation (37 CFR § 1.16):

	(Col. 1)	(Col. 2)	<u>SMALL ENTITY</u>		<u>OTHER THAN A SMALL ENTITY</u>	
<u>FOR:</u>	<u>NO. FILED</u>	<u>NO. EXTRA</u>	<u>RATE</u>	<u>FEE</u>	<u>RATE</u>	<u>FEE</u>
Basic Fee			\$380		\$760	\$760.00
Total Claims	39	19	\$ 9	\$	\$ 18	\$342.00
Indep Claims	6	3	\$ 39	\$	\$ 78	\$234.00
<input type="checkbox"/> Multiple Dependent Claims	1		\$130	\$	\$260	\$260.00
Total Filing Fee:				\$		\$1596.0

TOTAL FEES: \$1596.00

- ☒ A check including the amount of the above-indicated TOTAL FEES is attached.
- ☐ Please charge Deposit Account No.18-0020 in the amount of \$ _____.
- ☐ A check in the amount of \$ is attached.
- ☐ No fee is required.
- ☒ Conditional Petition for Extension of Time: An extension of time is requested in the present and/or the above-referenced parent application to provide for timely filing if an extension of time is still required after all papers filed with this transmittal have been considered.

- ☒ The Commissioner is hereby authorized to charge any underpayment of the following fees associated with this communication, including any necessary fees for extension of time, or credit any overpayment to Deposit Account No. 18-0020.
- ☒ Any filing fees under 37 CFR 1.16 including fees for the presentation of extra claims.
- ☒ Any parent application processing fees under 37 CFR 1.17.
- ☒ A **duplicate** copy of this sheet is attached for accounting purposes.

Respectfully submitted,

Dated: November 23, 1999

By: Barbara Rae-Venter

Barbara Rae-Venter, Ph.D.
Reg. No. 32,750

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BRV:JLW
Enclosures

PATENT

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In re application of: Goodman, *et al.*

) Examiner: Not Yet Assigned

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Filed: November 23, 1999

) **FIRST PRELIMINARY**

) **AMENDMENT**

)

For: **MOLECULAR FARMING**

)

BOX PATENT APPLICATION

Assistant Commissioner for Patents

Washington, D.C. 20231

Dear Sir:

Applicant is submitting herewith a First Preliminary Amendment in the above-referenced patent application. Prior to examination of the application, the Examiner is respectfully requested to enter the following amendments.

AMENDMENTS

In the Claims

1. (Amended) A method for producing [a] an expression product of a mammalian [peptide] viral pathogen gene which comprises:
growing plant cells containing an integrated sequence comprising,

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Jacob B. Buehly
(Signature)

Jacob Buehly
(Printed Name)

a first expression cassette having in the direction of transcription (1) a transcriptional and translational initiation region functional in said plant cells, (2) a mammalian viral pathogen structural gene [coding for said mammalian virus peptide], and (3) a termination region, whereby said structural gene is expressed to produce an expression product of said mammalian [peptide]viral pathogen gene; and

isolating said mammalian [peptide]viral pathogen gene expression product [substantially] free of non-plant cell [components] contaminants.

Cancel Claims 2-13.

Add the following new claims:

--14. (New) A method for producing an expression product of a mammalian viral pathogen gene, comprising:

growing a transformed dicotyledonous plant comprising plant cells containing an integrated sequence comprising a first expression cassette having in the direction of transcription (1) a first transcriptional and translational initiation region functional in said plant cells, (2) a first structural gene coding for said mammalian viral pathogen gene expression product, (3) a first termination region; and a second expression cassette having in the direction of transcription (4) a second transcriptional and translational initiation region functional in said plant cells, (5) a second structural gene coding for a peptide that allows selection of plant cells expressing said second structural gene, (6) a second termination region, so that said first structural gene is expressed and a mammalian viral pathogen gene expression product free of non-plant contaminants is obtained.

15. (New) An expression product of a mammalian viral pathogen gene produced according to the method of Claim 14.

16. (New) An expression product of a mammalian viral pathogen gene produced according to the method of Claim 14, further comprising:

the step of isolating said mammalian viral pathogen gene expression product from said plant.

17. (New) The expression product of a mammalian viral pathogen gene according to Claim 16, further comprising:

the step of purifying said expression product.

18. (New) An expression product of a mammalian viral pathogen gene according to Claim 1 or Claim 14, wherein said plant cells are seed cells.

19. (New) The expression product of a mammalian viral pathogen gene according to Claim 18, wherein said seed cells are rapeseed seed cells.

20. (New) An expression product of a mammalian viral pathogen gene according to Claim 1 or 14, wherein said plant cells are tobacco cells.

21. (New) An expression product of a mammalian viral pathogen gene according to Claim 14, wherein said first structural gene is expressed in a part of said plant which is edible.

22. (New) The expression product of a mammalian viral pathogen gene according to Claim 21, wherein said expression product has a physiological effect upon ingestion by a mammal.

23. (New) The method according to Claim 1 or Claim 14, wherein said integrated sequence contains one or more transcriptional and translational initiation region(s) derived at least in part from a transcriptional and translational initiation region of a Ti- or Ri-plasmid.

24. (New) The method according to Claim 23, wherein said transcriptional and translational initiation region is derived at least in part from a transcriptional and translational initiation region of a Ti- or Ri-plasmid regulates expression of mannopine synthase, octopine synthase or nopaline synthase.

25. (New) The method according to Claim 1 or Claim 14, wherein said transcriptional and translational initiation region of said first expression cassette regulates expression of a plant gene.

26. (New) The method according to Claim 1 or Claim 14, wherein said first or second expression cassette further comprises a T-DNA boundary.

27. (New) Plant matter comprising plant cells containing an expression product of a mammalian virus pathogen gene free from non-plant contaminants produced by a method comprising:

growing a transformed dicotyledonous plant comprising plant cells containing an integrated sequence comprising a first expression cassette having in the direction of transcription (1) a first transcriptional and translational initiation region functional in said plant cells, (2) a first structural gene coding for said expression product of a mammalian viral pathogen gene, (3) a first termination region; and a second expression cassette having in the direction of transcription (4) a second transcriptional and translational initiation region functional in said plant cells, (5) a second structural gene coding for a peptide that allows selection of plant cells expressing said second structural gene, (6) a second termination region, so that said first structural gene is expressed and plant matter comprising plant cells containing an expression product of a mammalian virus pathogen gene free of non-plant contaminants is obtained.

28. (New) The plant matter according to Claim 27, further comprising the step of isolating said expression product of mammalian viral pathogen gene substantially free of plant cell components.

29. (New) A composition comprising:
an expression product of a viral pathogen gene free from non-plant contaminants.

30. (New) The expression product of a mammalian viral pathogen gene according to Claim 29, wherein said expression product is a mature mammalian virus peptide.

31. (New) The expression product of a mammalian viral pathogen gene according to Claim 30, wherein said mature mammalian virus peptide is a mammalian virus coat peptide.

32. (New) The expression product of a mammalian viral pathogen gene according to Claim 30, wherein said mature mammalian virus peptide is a mammalian virus core peptide.

33. (New) Plant matter comprising:
plant cells containing a mammalian virus peptide.

34. (New) The plant matter according to Claim 33, wherein said plant matter is edible.

35. (New) The plant matter according to Claim 34, wherein said mammalian virus peptide has a physiological effect upon ingestion by a mammal.

36. (New) The plant matter according to Claim 33, wherein said mammalian virus peptide is a mammalian virus coat peptide.

37. (New) The plant matter according to Claim 33, wherein said mammalian virus peptide is a mammalian virus core peptide.

38. (New) The plant matter according to Claim 33, wherein said mammalian virus peptide is a mature mammalian virus peptide.

39. (New) Dicotyledonous plant cells having an integrated sequence comprising:

a first expression cassette having as operatively linked components in the direction of transcription (1) a first transcriptional and translational initiation region functional in said plant cells, (2) a first structural gene coding for a mammalian virus peptide, and (3) a first termination region.

40. (New) The dicotyledenous plant cells according to Claim 39, wherein said plant cells are tobacco plant cells.

41. (New) The dicotyledenous plant cells according to Claim 39, wherein said plant cells are seed cells.

42. (New) The dicotyledenous plant cells according to Claim 39, wherein said plant cells are rapeseed cells.

43. (New) The dicotyledenous plant cells according to Claim 39, wherein said mammalian virus peptide is a mammalian viral coat peptide.

44. (New) The dicotyledenous plant cells according to Claim 39, wherein said mammalian virus peptide is a mammalian viral core peptide.--

REMARKS

Claims 2-13 are cancelled and new Claims 14-44 have been added. Support for mammalian virus peptide is found on page 5, lines 1-3 and 21-26. Support for expression cassette is found on page 3, lines 17-29. Support for T-DNA and Ti- or Ri-plasmid is

Goodman, *et al.*

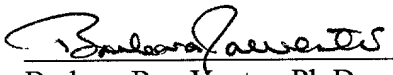
found on page 4, lines 7-10 and page 7, lines 1-27. Support for dicotyledonous plant is found on page 8, lines 3-13. Support for expression in edible plants and/or edible plant parts is found on page 9 line 32 through page 10, line 4. Support for expression in seeds or in rapeseed seeds is found on page 4, lines 29-33, and page 8, line 8. Support for tobacco is found on page 8, line 7 and page 17. Support for plant matter is found on page 3, lines 31-32, and on page 9, line 23.

CONCLUSION

In view of the above amendment and remarks, it is submitted that this application is now ready for allowance. Early notice to that effect is solicited. If in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney at (650) 328-4400.

Respectfully submitted,

Dated: November 23, 1999


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The undersigned has reviewed the chain of title and to the best of the undersigned's knowledge,
title is in the assignee identified above.

Date: November 23, 1999

By: Barbara Venter

Title: Attorney of Record
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MOLECULAR FARMING

BACKGROUND OF THE INVENTION5 Field of the Invention

10 An extensive literature has been developed demonstrating the ability to produce polypeptide sequences in a wide variety of cellular hosts. Numerous genes have been isolated from mammals and viruses, joined to transcriptional and translational initiation and termination regulatory signals from a source other than the structural gene and introduced into hosts in which the regulatory signals are functional. Frequently, the peptide of interest is prepared to be used for a physiological purpose. In many situations, physiological activity requires not only having the correct or substantially correct amino acid sequence, but the peptide must fold properly including proper disulfide linkage formation and may require further processing, such as acetylation, glycosylation or methylation.

20 For economic production, one would wish to use unicellular microorganisms, which could be grown in large fermentation tanks, do not have fastidious nutrient requirements and are relatively economical to maintain. Bacteria, such as E. coli, B. subtilis, or the like, fungi, such as yeast, Candida, filamentous fungi, or the like, offer economic opportunities to produce a wide variety of peptides. However, because of the substantial difference in the nature of the unicellular microorganisms and mammalian cells, the folding and processing in a mammalian cell appears to be substantially different from these lower order organisms. Therefore, the products which are obtained from the unicellular microorganisms may not have been properly processed or folded so as to realize a substantial proportion or all of the physiological

activity of the naturally occurring peptide obtained from a native host.

There therefore remains substantial interest in providing alternative economic systems for producing peptides, where high yields may be obtained and significantly, the products may be produced in a form providing for a high degree of physiological activity common to the wild-type peptide having the same or substantially the same amino acid sequence.

Brief Description of the Relevant Literature

References concerned with expression of various interferons include Goeddel et al., Nucleic Acids Res. (1980) 8:4057-4074; Goeddel, Nature (1980) 287:411-415; Yelverton et al., Nucleic Acids Res. (1981) 9:731-741; Gray et al., Nature (1982) 295:503-508; Devos et al., Nucleic Acids Res. (1982) 10:2487-2501; Grey and Goeddel, Proc. Natl. Acad. Sci. USA (1983) 80:5842-5846; Scahill et al., Proc. Natl. Acad. Sci. USA (1983) 80:4654-4658. See also, Horsch et al., Science (1985) 227:1229-1231.

Wide host range cloning vectors are described by Knauf and Nester, Plasmid (1982) 8:45-54. The nucleotide sequence of the T-DNA region is described by Barker et al., Plant Molecular Biology (1983) 2:335-350. See also, EPA 0 116 718 and PCT WO84/02913.

SUMMARY OF THE INVENTION

Efficient production of physiologically active mammalian proteins is provided by introducing functional constructs containing the mammalian structural gene into a plant cell. The construct is able to express the desired peptide in an isolatable form. The plant cells may be grown in culture or cultivated in an appropriate nutrient medium or soil and the mammalian protein harvested. Particularly, T-DNA transformation may be employed for integration of

the construct into the plant genome under conditions where the cells can be used to produce plants.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

5 Novel DNA sequences are provided comprising constructs functional in plant cells for the expression of physiologically active mammalian peptides. The constructs provide for functional transcriptional and translational initiation and termination regulatory
10 signals which with the construct integrated into the plant genome, provide for efficient expression of the structural gene. Depending upon whether the cells are grown in culture or cultivated in soil or other medium, these cells or plants may be harvested, and the desired
15 product extracted and purified in accordance with conventional techniques.

 The construct for introduction into the plant will be considered first. While the construct may be either DNA or RNA, for the most part, the construct
20 will be DNA even though the construct may be ultimately introduced into the plant cell as RNA, such as a virus. One significant element of the construct will be the transcriptional cassette involving the transcriptional initiation region, which may be divided into a
25 regulatory domain and the usually proximal RNA polymerase binding domain, the structural gene coding for the mammalian peptide of interest, and a terminator region which provides for termination of transcription and translation. Another significant element is the
30 regulation of expression at a particular stage of differentiation, in a particular plant part, e.g., roots, leaves, stalk, or the like. In many situations a polypeptide leader signal will be desirable which directs the product to a particular organelle. This
35 will be of substantial significance where the organelle may be involved in the proper processing of the peptide.

A wide variety of transcriptional initiation regions may be employed, which are inducible, e.g., tissue specific, or constitutive. The transcriptional initiation regions may come from the tumor-inducing plasmids of Agrobacterium, particularly the genes associated with T-DNA, or from viruses, or plants. Among the T-DNA transcription initiation regions which may find use include those regions associated with octopine synthase, nopaline synthase, mannopine synthase, transcript 7, and the like. Among viral transcription initiation regions are included the caulimovirus full length promoter and the region VI promoter. Among plant transcription initiation regions are the ribulose-1,5-bisphosphate carboxylase small subunit region, which is light inducible, or the napin or other seed protein region, for formation in seed.

The transcription initiation regions may be isolated from their natural sites in a host or may be sequenced and synthesized so as to have the same or substantially the same sequence as the wild-type region. Where inducible regulation is desired, domains may be obtained from different sources, so that a regulatory domain may be obtained from one source and joined to an RNA polymerase binding domain from a different source. In this manner, one may provide for the use of strong RNA polymerase binding domain associated with one structural gene, while having a regulatory domain associated with a different structural gene. These hybrid regulatory regions may find particular use where one wishes to induce the production of the desired gene at a particular phase in the growth of the plant or to have the product located in a particular plant part, such as seed, leaves, or the like. Any transcriptional termination regulatory region may be used which is functional in plants, e.g., prokaryotic or eukaryotic, from T-DNA, plants, viruses or mammals.

The structural gene may be any mammalian gene of interest, which includes mammalian viral pathogen genes. A wide variety of genes have been isolated and shown to be capable of production in unicellular micro-organisms, to various degrees of biological activity and efficiencies, and in mammalian cells, with the ever present concern that the mammalian cells are transformed cells, so that any product must be carefully purified to ensure the complete absence of any nucleic acid contaminant. Structural genes of interest include α -, β - and γ -interferons, immunoglobulins, with the structural genes coding for the light and heavy chains and desirably assembly occurring in the plant cell, lymphokines, such as interleukins 1, 2 and 3, growth factors, including insulin-like growth factor, epidermal growth factor, platelet derived growth factor, transforming growth factor- α , - β , etc., growth hormone, insulin, collagen plasminogen activator, blood factors, such as factors I to XII, histocompatibility antigens, enzymes, or other mammalian proteins, particularly human proteins.

Among the antigens associated with viral pathogens, include the core and envelope proteins of leukemia and lymphotropic retroviruses, such as HTLV-I, -II and -III, feline leukemia virus, etc., surface antigens of herpes simplex virus, hepatitis B virus, adenovirus, and the like.

Peptides of interest other than those indicated above will be peptides which may be administered physiologically, where growth in plants diminishes the probability of contaminants causing an adverse response upon administration to a mammalian host.

Plants may also find use in preparing other proteins than to be administered to a host, where the mammalian protein, such as an enzyme, may require folding and/or processing that is unavailable in unicellular microorganisms. Not only may plants be used to prepare the mature peptide, but in many

instances it may be desirable to prepare the precursor, which may require cleavage and assembly, either endogenous or exogenous to the plant. Peptides can be prepared having a naturally occurring transit or leader peptide or a transit or leader peptide from a plant peptide. The transit peptide will serve to subject the entire peptide product to the processing and maturing of the peptide. Such processing may include specific peptide cleavage, e.g., removal of the transit peptide, glycosylation at glycosylation sites, folding with appropriate formation of disulfide linkages.

Any convenient terminator may be employed which provides for efficient termination in conjunction with a particular transcriptional initiation region. The terminator may be the terminator region normally associated with the transcriptional initiation region or associated with a different transcriptional initiation region, so long as the region is functional in plants.

In order to select for plant cells that have successfully integrated the construct, the expression construct or cassette will usually be joined to a marker. The marker will allow for either screening or selection, usually selection. A number of different antibiotic resistance genes are known that can find use in plants to serve as markers. These genes include enzymes providing resistance to kanamycin, chloramphenicol, G418, gentamycin, and the like. These genes will have transcriptional and translational initiation and termination regulatory regions that may be the same or different from the regions employed for the structural gene of interest. Usually constitutive expression will be provided, rather than inducible expression.

In addition to the cassette and marker, depending upon the manner in which the DNA construct will be introduced into the plant cell, other sequences may be necessary. Where the Agrobacterium tumor-

inducing system is to be employed, one or both of the T-DNA boundaries of a Ti- or Ri-plasmid will be present, particularly the right boundary region. Each boundary region will generally be of about 1 to 1.5kbp.

5 The DNA construct of the cassette, marker and T-DNA may then be employed in a variety of ways. For integration into a Ti- or Ri-plasmid, the construct may be introduced into an appropriate Agrobacterium strain carrying the tumor-inducing plasmid, whereby the
10 construct will become integrated into the tumor-inducing plasmid and may then be transferred to plant cells. Alternatively, the construct may be joined to a broad spectrum replication system, such as a P1 incompatibility replication system and transformed into an Agrobacterium
15 containing an armed or disarmed tumor-inducing plasmid. Integration will occur and transfer to the plant cell of the construct along with other genes and markers. When transfer is with an armed tumor-inducing plasmid (Ti or Ri T-DNA containing plasmid) genes conferring
20 tumor formation will be transferred, so that galls may form. With disarmed tumor plasmids (lacking T-DNA) the tumor-causing genes cannot be transferred and gall formation is not encountered. A transposon may be employed containing the construct and the gene coding
25 for transposase, such as in the Ac-Ds system. A viral system may be employed which provides for integration into the host genome.

Transfer of the DNA construct into the plant cell may be by infection with A. tumefaciens or A.
30 rhizogenes, microinjection, liposome fusion, viral infection, or the like. The particular manner in which the DNA is introduced into the plant cell for integration is not critical to this invention.

Usually, the construct will be joined to a
35 prokaryotic replication system, for example, a system functional in E. coli, so as to allow for cloning at the various stages of preparation of the construct. A

wide variety of replication systems are available, both plasmid and phage, such as ColE1, λ , etc.

Plant cells which are employed may be either monocots or dicots and will be chosen in accordance with the manner in which the desired gene is to be produced and harvested. Plants which may find use include tobacco, sunflower, corn, sugar cane, soybean, tomato, alfalfa, mustard, sugar beet, rapeseed, etc. The product may be found in plant parts such as seed, leaves, fruit, roots, stalks, tubers, or combinations thereof. Thus, the peptide of interest may be the sole purpose for growing the plant or be an additional product.

The construct will be prepared in conventional ways. DNA sequences may be detected by employing probes, which may be designed based on known amino acid sequences or prior isolation of all or fragments of mRNA or chromosomal DNA. The sequences may be restriction mapped or sequenced and the entire gene obtained by various techniques, such as walking, using a plurality of primers, or the like. Once the sequence has been isolated, it may be ligated to other sequences, either directly or through linkers, where the linkers may provide no or portions of coding sequences. Various strategies may be devised based on available restriction sites, the absence of restriction sites, the ability to introduce restriction sites, the availability of particular fragments, the presence of sequences which require excision, and the like. The particular strategy will be dependent upon the gene which is employed, the particular regulatory systems, the availability of vectors having one or more of the desired sequences, as well as other practical considerations.

The manner in which the construct is introduced into plants may be varied widely. This has already been indicated by virtue of the different

sequences which may be included in the construct. Of particular interest is the presence of T-DNA for integration in conjunction with the vir genes of the Ti-plasmid which may be present on a plasmid other than the plasmid containing the foreign gene. Thus, the necessary genetic capability for integration into the plant cell can be provided, in conjunction with infection with Agrobacterium or introduction of the DNA by other means. Descriptions of introduction of DNA into plants may be found in Pedersen et al., Plant Cell Reports (1983) 2:201-204; Hooykaas-Van Slogteren et al., Nature (1984) 311:763-764; de Cleene and de Ley, The Botanical Review (1976) 42:389-466, and references cited therein, are incorporated herein by reference.

The transformed plant cells will then be grown in appropriate nutrient medium to provide for selected calli, where plant cells or protoplasts have been modified. Once the calli has formed, the medium may then be changed to encourage root and shoot formation and the resulting shoots transferred to appropriate growth medium for growth of plants. When the plants have been grown to the desired stage, the plants or plant parts, e.g., seeds, fruit or the like, may be harvested, and the desired product isolated in accordance with conventional ways. Thereafter, the gene may be regenerated from seeds, so that the process of regeneration from calli need not be repeated. The plant may be ground and extracted with appropriate solvents, chromatographed, crystallized, solvent extracted, etc. The crude product may then be purified in accordance with the nature of the product.

In some instances it may be neither necessary nor desirable to extract and isolate the mammalian protein product from the plant. Where the product can have a physiological effect on ingestion, it may be sufficient that the product be retained with the plant. This will be true where the plant part is edible, such

as fodder which could include nutritional qualities, such as bovine growth hormone, seed, nuts, fruit, and vegetables, which could include proteins involved in the regulation of digestion, or the like.

5 The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

10 The HindIII-SmaI fragment of Tn5 containing the entire structural gene for APHII (Jorgensen et al., Mol. Gen. (1979) 177:65) was cloned into pUC8 (Vieira and Messing, Gene (1982) 19:259), converting the fragment into a HindIII-EcoRI fragment, since there is an EcoRI site immediately adjacent to the SmaI site.

15 The PstI-EcoRI fragment containing the 3'-portion of the APHII gene was then combined with an EcoRI-BamHI-SalI-PstI linker into the EcoRI site of pUC7 (pCGN546W). Since this construct does not confer kanamycin resistance, kanamycin resistance was obtained

20 by inserting the BglIII-PstI fragment of the APHII gene into the BamHI-PstI site (pCGN546X). This procedure reassembles the APHII gene, so that EcoRI sites flank the gene. An ATG codon was upstream from and out of reading frame with the ATG initiation codon of APHII.

25 The undesired ATG was avoided by inserting a Sau3A-PstI fragment from the 5'-end of APHII, which fragment lacks the superfluous ATG, into the BamHI-PstI site of pCGN546W to provide plasmid pCGN550.

30 The EcoRI fragment containing the APHII gene was then cloned into the unique EcoRI site of pCGN451, which contains an octopine synthase cassette for expression, to provide pCGN552.

35 pCGN451 includes an octopine cassette which contains about 1556bp of the 5' non-coding region fused via an EcoRI linker to the 3' non-coding region of the octopine synthase gene of pTiA6. The pTi coordinates are 11,207 to 12,823 for the 3' region and 13,643 to

15,208 for the 5' region as defined by Barker et al.,
Plant Mol. Biol. (1983) 2:325.

The 5' fragment was obtained as follows. A small subcloned fragment containing the 5' end of the coding region, as a BamHI-EcoRI fragment was cloned in pBR322 as plasmid pCGN407. The BamHI-EcoRI fragment has an XmnI site in the coding region, while pBR322 has two XmnI sites. pCGN407 was digested with XmnI, resected with Bal31 nuclease and EcoRI linkers added to the fragments. After EcoRI and BamHI digestion, the fragments were size fractionated, the fractions cloned and sequenced. In one case, the entire coding region and 10bp of the 5' non-translated sequences had been removed leaving the 5' non-transcribed region, the mRNA cap site and 16bp of the 5' non-translated region (to a BamHI site) intact. This small fragment was obtained by size fractionation on a 7% acrylamide gel and fragments approximately 130bp long eluted. This size fractionated DNA was ligated into M13mp9 and several clones sequenced and the sequence compared to the known sequence of the octopine synthase gene. The M13 construct was designated p14, which plasmid was digested with BamHI and EcoRI to provide the small fragment which was ligated to a XhoI to BamHI fragment containing upstream 5' sequences from pTiA6 (Garfinkel and Nester, J. Bacteriol. (1980) 144:732) and to an EcoRI to XhoI fragment containing the 3' sequences. The resulting XhoI fragment was cloned into the XhoI site of a pUC8 derivative, designated pCGN426. This plasmid differs from pUC8 by having the sole EcoRI site filled in with DNA polymerase I, and having lost the PstI and HindIII site by nuclease contamination of HincII restriction endonuclease, when a XhoI linker was inserted into the unique HincII site of pUC8. The resulting plasmid pCGN451 has a single EcoRI site for the insertion of protein coding sequences between the 5' non-coding region (which contains 1,550bp of 5'

non-transcribed sequence including the right border of the T-DNA, the mRNA cap site and 16bp of 5' non-translated sequence) and the 3' region (which contains 267bp of the coding region, the stop codon, 196bp of 3' non-translated DNA, the polyA site and 1,153bp of 3' non-transcribed sequence). pCGN451 also provides the right T-DNA border.

The resulting plasmid pCGN451 having the ocs 5' and the ocs 3' in the proper orientation was digested with EcoRI and the EcoRI fragment from pCGN551 containing the intact kanamycin resistance gene inserted into the EcoRI site to provide pCGN 552 having the kanamycin resistance gene in the proper orientation.

This ocs/KAN gene was used to provide a selectable marker for the trans type binary vector pCGN587.

The 5' portion of the engineered octopine synthase promoter cassette consists of TiA6 DNA from the XhoI at bp 15208-13644 (Barker's numbering), which also contains the T-DNA boundary sequence (border) implicated in T-DNA transfer. In the plasmid pCGN587 the ocs/KAN gene from pCGN552 provides a selectable marker as well the right border. The left boundary region was recloned from the HindIII-EcoRI fragment as a KpnI-EcoRI fragment in pCGN565 to provide pCGN580. pCGN565 is a cloning vector based on pUC8-Cm, but containing pUC18 linkers. pCGN580 was linearized with BamHI and used to replace the smaller BglII fragment of pVCK102 (Knauf and Nester, Plasmid (1982) 8:45), creating pCGN585. By replacing the smaller SalI fragment of pCGN585 with the XhoI fragment from pCGN552 containing the ocs/KAN gene, pCGN587 was obtained.

pCGN807 is derived from pBR322, contains the tetracycline resistance gene, has about 300bp of a sequence including the trp promoter 5' to a 750bp sequence including all of the murine γ -interferon

structural cDNA and a 3'-untranslated region, with the gene described in Gray and Goeddel, Proc. Natl. Acad. Sci. USA (1983) 80:5842-5846. The promoter and gene are an EcoRI fragment of about 834bp. pCGN807 was
 5 digested with EcoRI and HpaI which fragment was inserted into pEMBL18+, which had been digested with EcoRI and SmaI creating pCGN808. pEMBL18+ (Dente et al., Nucleic Acids Res. (1983) 11:1645) differs from the pEMBL plasmids in having the pUC18 linker inserted
 10 into the β -galactosidase gene. pCGN808 was digested with EcoRI and BamHI to provide a 540bp fragment which was inserted into pCGN46 which had been digested with EcoRI and BamHI to provide for the murine γ -interferon cDNA gene to be in the proper orientation in relation
 15 to the mannopine synthase (mas) 5' transcription initiation region and the ocs 3' transcription termination region (pCGN809).

An approximately 5.5kbp EcoRI fragment (EcoI3 or EcoC) carrying a portion of the T-R DNA (Barker et al., Plant Mol. Biol. (1983) 2:325) including the
 20 mannopine synthase promoter region (P_{MAS}) was cloned in a vector designated pVK232. After digestion of pVK232 with EcoRI, EcoI3 was inserted into the EcoRI site of pACYC184 to yield plasmid pCGN14. pCGN14 was digested
 25 with SphI and ClaI (respectively at position 21562 and 20128 of the Barker et al. sequence, supra) to remove the P_{MAS} region which was inserted into pUC19 (Pharmacia, Inc.) which had been digested with SphI and AccI to yield pCGN40. The P_{MAS} region includes a ClaI
 30 recognition site internally which is methylated, so as to resist digestion.

pCGN40 was digested with EcoRV and EcoRI where the EcoRV site is in the T-DNA, while the EcoRI site is in the polylinker of pUC19 to provide a
 35 fragment having the P_{MAS} region. pCGN451 containing the octopine synthase cassette was digested with SmaI and EcoRI and the larger fragment isolated from which

the octopine synthase 5' region had been removed. The EcoRV-EcoRI P_{MAS} region was substituted into pCGN451 for the octopine synthase 5' region, where the transcriptional initiation and termination regions were separated by a polylinker to provide pCGN46.

To introduce the plasmid pCGN809 onto the wide host range T-DNA binary vector pCGN587, pCGN809 was transformed into an E. coli polA1 mutant containing pCGN587. The two plasmids pCGN587 and pCGN809 share two regions of homology where recombination can occur: at the ocs 3' regions of the APHII gene and the γ -interferon gene, and at the pUC origins of the two plasmids, recombination at either site results in a plasmid containing both the γ -interferon gene and the APHII gene as intact expression units. In the polA1 mutant, the pCGN809 plasmid, which has a pUC origin of replication, can only survive by integration into the pCGN587 plasmid, which has a wide host range replication system. Since the pCGN809 construct has ampicillin resistance, the chimeric plasmid pCGN810 can be selected. The transformed E. coli host was grown in a nutrient medium containing 100 μ g/ml ampicillin and surviving clones isolated. Plasmid pCGN810 was then mated into A. tumefaciens C58 (Watson et al., J. Bacteriol. (1974) 123:255) by the method of Ditta et al., Proc. Natl. Acad. Sci. USA (1978) 77:7347. pCGN810 was also mated into A. tumefaciens K61, which contains a disarmed Ti-plasmid, as compared to C58 which has the genetic capability for nopaline expression, and is an armed Ti-plasmid. The transformed Agrobacterium was selected with carbenicillin (50 μ g/ml) and streptomycin (150 μ g/ml) at 30°C for two to three successive streak outs. After growth under selection, DNA was isolated and restricted with BamHI and EcoRI and verified by probe hybridization. The cloned pCGN808 was used as the nick-translated probe and confirmed the presence of a

565bp γ -interferon fragment in both the expression vector pCGN46 and the T-DNA plasmid pCGN587 in armed and disarmed Agrobacterium.

Four pCGN810 constructs in both C58 and K61
5 were cocultivated with Nicotiana tabacum xanthii protoplasts and clumps of transformed cells which were selected in 100 μ g/ml kanamycin were transferred to solid media containing kanamycin (100 μ g/ml) for further growth and regeneration into plants.

10 Tobacco leaf disks were cocultivated with pCGN810 in C58 and K61 Agrobacterium strains and the disks transferred to selective media with kanamycin (100 μ g/ml) for growth to callus.

Sterile tobacco plants were stem-stab
15 inoculated with A. tumefaciens C58 (pCGN810) resulting in the production of gall tissue which was excised and transferred onto solid media containing kanamycin (100 μ g/ml). After considerable callus proliferation, most of the gall-induced callus material was harvested
20 for mRNA and protein analysis. A small portion of the gall-callus material started to shoot on the kanamycin medium without hormones. Once transferred to shooting media, the shoot development looked normal for most of the plantlets, though some were teratoma-like. The
25 plantlets were transferred to rooting medium containing kanamycin (100 μ g/ml) and most of the morphology normal plantlets rooted.

The rest of the gall-induced callus material was pooled and used for preliminary expression analysis
30 for γ -interferon as compared with control callus (plant cells transformed with A. tumefaciens C58(pCGN587)). After extraction of the plant material and immunoprecipitation with antisera against γ -interferon, the extract was electrophoresed on 15% SDS-PAGE,
35 electroblotted to nitrocellulose and blocked with γ -interferon antisera. Radioactively labeled S. aureus protein A was hybridized to the filter, so as to bind

to any immune complexes which had formed. In the transformed plant tissue (A. tumefaciens C58 (pCGN810)), a visible band ran at the same size as the positive control γ -interferon samples.

5 Analysis of the mRNA obtained after an oligo-dT prep of total RNA was performed using nick-translated isolated gene fragments to the Tn5 kanamycin gene (in pCGN587) and the γ -interferon fragment (in pCGN808). The Northern data showed a
10 message in both pCGN587 and pCGN810 mRNA preps which hybridize to the kanamycin fragment. Only the transformed pCGN810 sample, hybridized to the γ -interferon gene probe showing two mRNA species, each terminating in a different sized noncoding 3'-sequence.

15 Samples were prepared and bioassayed for murine γ -interferon. The samples were prepared as follows: To frozen tobacco tissue from tissue culture (1.6 or 1.0g) ground in liquid nitrogen was added 5 or 2.5ml, respectively, extraction buffer (1M NaCl,
20 25mg/ml BSA, 10mM DTT, 10mM cysteine, 20mM NaPO₄, pH 7.4) and grinding continued until the mixture was liquid. The liquid was then centrifuged twice at 16K rpm for 20min, transferring the liquid to a fresh tube after the first separation. The sample was then
25 diluted to 5ml and 0.5ml control pore glass beads (CPG) (washed and autoclaved) were added and the mixture agitated at 4°C for 4hr. The mixture was then loaded onto a silane treated Pasteur pipette column, the column washed with 3ml 20mM NaPO₄, 1M NaCl and the
30 product eluted with 5ml 1M NaCl, 30% ethylene glycol. The material eluted from the column was then dialyzed overnight against 20mM NaPO₄, 1M NaCl. Dialysis buffer was changed once at 3hr. The dialysed sample was concentrated with a Millipore immersible-CX 10,000
35 ultrafilter to less than about 1ml and then a series of dilutions with BSA buffer (1M NaCl, 20mM NaPO₄, pH 7.4, 25mg/ml BSA) performed. Comparison samples were made

from transformed and untransformed tobacco, where no γ -interferon gene was introduced, where the tissue was or was not supplemented with γ -interferon. The following table indicates the results.

5

TABLE

	Tissue ¹	Dilution ²	γ -IFN activity ³
10	UT A	0 1 2 3	9 9 9 9
15	UT B	0 1 2 3	741 188 15 9
20	T A	0 1 2 3	9 9 9 9
	T B	0 1 2 3	9191 9191 1042 261
25	T- γ IFN	0 1 2 3	197 41 9 9

30 ¹ UT - untransformed tobacco tissue
 T - tobacco tissue transformed with foreign DNA
 T- γ IFN - tobacco tissue transformed with the murine γ -IFN gene

A - no murine γ -IFN added
 B - 5 μ g γ -IFN added to 5ml of sample prior to purification treatment

35 ² 0 = 0.5; 1 = 10⁻¹; 2 = 10⁻²; 3 = 10⁻³

³ Assay procedure is described in Yip et al.,
Proc. Natl. Acad. Sci. USA (1982) 79:1820-1824.

The above results demonstrate that one can produce physiologically active mammalian proteins in plant cells. Stable messages are produced by transcription of the mammalian gene integrated into the plant genome, which message can then be translated into the mammalian product, so as to provide physiologically active proteins. The mammalian proteins can be readily isolated free of deleterious contaminants, such as oncogenic DNA, exotoxins, and the like. In addition, the products can be processed to provide for a mature product having the same or substantially the same structure and composition as the naturally-occurring peptide product.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method for producing a mammalian peptide which comprises:

growing plant cells containing an integrated
5 sequence comprising,

a first expression cassette having in the direction of transcription (1) a transcriptional and translational initiation region functional in said plant cells, (2) a structural gene coding for said
10 mammalian peptide, and (3) a termination region,

whereby said structural gene is expressed to produce said mammalian peptide; and

isolating said mammalian peptide substantially free of plant cell components.
15

2. A method according to Claim 1, wherein said integrated sequence ~~[includes]~~ ^{comprise} a second expression cassette having in the direction of transcription (1) a transcriptional and translational initiation region
20 functional in said plants, (2) a structural gene coding for a peptide which allows for selection of plant cells expressing said peptide, and (3) a termination region.

3. A method according to Claim 2, wherein
25 said transcriptional and translational initiation region ^{-- of said first expression cassette --} is derived at least in part from a transcriptional and translational initiation region of a Ti- or Ri-plasmid.

30 4. A method according to Claim 3, wherein said transcriptional and translational initiation region ^{-- of said first expression cassette --} regulates expression of mannopine synthase, octopine synthase or nopaline synthase.

35 5. A method according to Claim 1, wherein said transcriptional and translational initiation

region of said first expression cassette regulates expression of a plant gene.

6. A method according to Claim 1, wherein
5 said integrated sequence [includes] ^{--comprises--} a boundary region from T-DNA.

7. A method for producing an interferon which comprises:
10 growing plant cells containing an integrated sequence comprising,
a first expression cassette having in the direction of transcription (1) a transcriptional and translational initiation region functional in said
15 plant cells and derived from a region which regulates expression of a T-DNA gene, (2) a structural gene coding for an interferon, and (3) a termination region functional in said plant cells,
whereby said structural gene is expressed to
20 produce said interferon, and
isolating said interferon substantially free of plant cell components.

⁸ dicotyledon plant cells A method according to Claim 7, wherein[^]
25 said integrated sequence [includes] ^{--comprises--} a second expression cassette having in the direction of transcription (1) a transcriptional and translational initiation region functional in said plant cells, (2) a structural gene coding for an enzyme which imparts antibiotic
30 resistance, and (3) a T-DNA boundary.

9. A method according to Claim 8, wherein said first expression cassette transcriptional and translational initiation region regulates expression of
35 the mannopine synthase gene of T-DNA.

10. An expression cassette comprising a DNA sequence having in the direction of transcription a transcriptional and translational initiation region functional in plant cells, a structural gene coding for a mammalian peptide, and a termination region functional in plant cells.

11. An expression cassette according to Claim 10 including joined to said DNA sequence a second expression cassette comprising a second transcriptional and translational initiation region functional in plant cells, a structural gene coding for a peptide providing a phenotypic property capable of selection in plant cells, and a termination region functional in plant cells.

12. An expression cassette according to Claim 11, ~~including~~ ^{comprising} a T-DNA boundary.

13. A DNA construct comprising a first expression cassette having in the direction of transcription a ^{mannopine synthase} transcriptional and translational initiation region regulating the expression of mannopine synthase of T-DNA, a structural gene coding for γ -interferon and a termination region functional in plant cells, a second expression cassette comprising in the direction of transcription a transcriptional and translational initiation region regulatory the expression of octopine synthase of T-DNA, a structural gene coding for an enzyme imparting antibiotic resistance to plant cells, and a termination region functional in plant cells, and a T-DNA boundary.

MOLECULAR FARMING

ABSTRACT OF THE DISCLOSURE

5 Novel constructs are provided for expression
of physiologically active mammalian proteins in plant
cells, either in culture or under cultivation. The
constructs provide a promoter functional in a plant
host, a structural gene coding for mammalian protein
10 and a terminator functional in a plant host. The
construct is introduced into a plant cell to become
integrated into the plant genome for expression in the
plant cells or plants. The plant cells may be
harvested and the mammalian protein isolated in
15 physiologically active form.

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30

35

PATENT

ATTORNEY DOCKET NO. CGNE.023.03US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Goodman, *et al.*

Serial No.: 08/164,346

Filed: December 8, 1993

For: **MOLECULAR FARMING**

) Examiner: D. Fox

) Art Unit: 1803

) **REVOCATION AND**
) **APPOINTMENT OF NEW POWER**
) **OF ATTORNEY UNDER**
) **37 C.F.R. § 3.73(b)**

Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

The undersigned, an authorized representative of Calgene, Inc., having a place of business at 1920 Fifth Street, Davis, CA 95616, and being assignee of 100% of the entire right, title and interest in the above-described patent application by virtue of an Assignment.

[] enclosed herewith.

☒ recorded by the U.S. Patent & Trademark Office at Reel 4480, Frame 0482

hereby revokes all powers of attorney previously granted in this application and hereby appoints:

BARBARA RAE-VENTER, Ph.D., Reg. No. 32,750
JAMES A. BRADBURN, Ph.D., Reg. No. 38,389
TIMOTHY L. SMITH, Ph.D., Reg. No. 35,367
DONNA E. SCHERER, Reg. No. 34,719
CARL J. SCHWEDLER, Reg. No. 36,924

as attorneys/agents with full power of substitution, association and revocation, to prosecute this application and to transact all business in the United States Patent and Trademark Office connected therewith, and hereby requests that all correspondence and telephone communications about the application be addressed to:

CERTIFICATE OF TRANSMISSION BY FACSIMILE

I hereby certify that this correspondence is being transmitted by facsimile machine to Examiner D. Fox, Art Unit 1803, Facsimile Number (703) 308-4227, at the United States Patent and Trademarks Office, Washington, D.C. 20231 on 4/26/96

(Signature)

(Printed Name)

RAE-VENTER AND ASSOCIATES
P.O. Box 60039
Palo Alto, CA 94306


The undersigned has reviewed all the documents in the chain of title of the patent application identified above and, to the best of the undersigned's knowledge and belief, title is in the assignee identified above.

The undersigned (whose title is supplied below) is empowered to act on behalf of the assignee.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Respectfully submitted,

Dated: 4/8/96



Lloyd Kunimoto
Vice President of Corporate Development

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C:\BRV\DATA\CALGENE\4233\REV&POA22196

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

MOLECULAR FARMING

the specification of which ☒ is attached hereto or ☐ was filed on _____ as Application Serial No. _____ and was amended on _____ (if applicable).

I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a). I claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)

COUNTRY	APPLICATION NUMBER	DATE OF FILING	PRIORITY CLAIMED UNDER 35 U.S.C. 119
			Yes _____ No _____
			Yes _____ No _____

I claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION SERIAL NO.	DATE OF FILING	STATUS
		<input type="checkbox"/> Patented <input type="checkbox"/> Pending <input type="checkbox"/> Abandoned
		<input type="checkbox"/> Patented <input type="checkbox"/> Pending <input type="checkbox"/> Abandoned

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) who are partners and associates in the firm of Townsend and Townsend to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

Bertram I. Rowland, Reg. No. 20,015

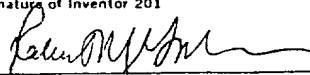
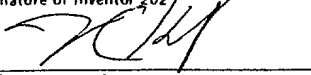
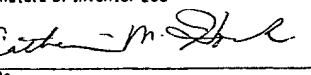
William M. Smith, Reg. No. 30,223

Stephen L. Hurst, Reg. No. 31,266

SEND CORRESPONDENCE TO:	Bertram I. Rowland TOWNSEND and TOWNSEND Steuart Street Tower, One Market Plaza San Francisco, CA 94105	DIRECT TELEPHONE CALLS TO: (name, registration number, and telephone number) Bertram I. Rowland Reg. No. 20,015 <input type="checkbox"/> (415) 543-9600 or <input checked="" type="checkbox"/> (415) 493-2590
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201	FULL NAME OF INVENTOR	Last Name GOODMAN	First Name Robert	Middle Name or Initial M.
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203	FULL NAME OF INVENTOR	Last Name HOUCK	First Name Catherine	Middle Name or Initial M.
	RESIDENCE & CITIZENSHIP	City Vacaville	State or Foreign Country California	Country of Citizenship U.S.A.
	POST OFFICE ADDRESS	Post Office Address 172 Carmel Court	City Vacaville	State or Country California

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature of Inventor 201 	Signature of Inventor 202 	Signature of Inventor 203 
Date 8-1-85	Date 8/1/85	Date 8-1-85

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

MOLECULAR FARMING

the specification of which ☒ is attached hereto or ☐ was filed on _____ as Application Serial No. _____ and was amended on _____ (if applicable).

I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a). I claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)

COUNTRY	APPLICATION NUMBER	DATE OF FILING	PRIORITY CLAIMED UNDER 35 U.S.C. 119
			Yes_____ No_____
			Yes_____ No_____

I claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION SERIAL NO.	DATE OF FILING	STATUS
		<input type="checkbox"/> Patented <input type="checkbox"/> Pending <input type="checkbox"/> Abandoned
		<input type="checkbox"/> Patented <input type="checkbox"/> Pending <input type="checkbox"/> Abandoned

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) who are partners and associates in the firm of Townsend and Townsend to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

Bertram I. Rowland, Reg. No. 20,015

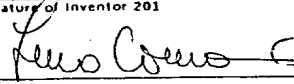
William M. Smith, Reg. No. 30,223

Stephen L. Hurst, Reg. No. 31,266

SEND CORRESPONDENCE TO:	Bertram I. Rowland TOWNSEND and TOWNSEND Steuart Street Tower, One Market Plaza San Francisco, CA 94105	DIRECT TELEPHONE CALLS TO: (name, registration number, and telephone number) Bertram I. Rowland Reg. No. 20,015 <input type="checkbox"/> (415) 543-9600 or <input checked="" type="checkbox"/> (415) 493-2590
-------------------------	--	---

201	FULL NAME OF INVENTOR	Last Name COMAI	First Name Luca	Middle Name or Initial
	RESIDENCE & CITIZENSHIP	City Davis	State or Foreign Country California	Country of Citizenship Italy
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			Zip Code 95616	
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	RESIDENCE & CITIZENSHIP	City	State or Foreign Country	Country of Citizenship
	POST OFFICE ADDRESS	Post Office Address	City	State or Country
			Zip Code	
203	FULL NAME OF INVENTOR	Last Name	First Name	Middle Name or Initial
	RESIDENCE & CITIZENSHIP	City	State or Foreign Country	Country of Citizenship
	POST OFFICE ADDRESS	Post Office Address	City	State or Country
			Zip Code	

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature of Inventor 201 	Signature of Inventor 202	Signature of Inventor 203
Date 8-1-85	Date	Date

PATENT

ATTORNEY DOCKET NO. CGNE.023.08US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Goodman, *et al.*)
) Examiner: Not Yet Assigned
Serial No.: Not Yet Assigned)
) Art Unit: Not Yet Assigned
Filed: November 23, 1999)
)
For: Molecular Farming) **REVOCATION AND**
) **APPOINTMENT OF NEW POWER**
) **OF ATTORNEY**

Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

The undersigned, an authorized representative of Calgene, Inc., having its principal place of business at 1920 Fifth Street, Davis, California 95616, being assignee of 100% interest of this application, the Assignment being recorded at Reel 4480, Frame 0482, hereby revokes all powers of attorney previously granted in this application and hereby appoints

Barbara Rae-Venter	Reg. No. 32,750
Bertram I. Rowland	Reg. No. 20,015
Carl Schwedler, Esq.	Reg. No. 36,924

as attorneys with full power of substitution and revocation to prosecute this application and to transact all business in the United States Patent and Trademark Office connected therewith, and hereby request that all correspondence regarding this application be sent to the firm of:

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